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<110> F. HOFFMANN-LA ROCHE AG

<120> PROCESS FOR THE MANUFACTURE OF CAROTENOIDS AND
BIOLOGICALLY USEFUL MATERIALS THEREOF

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<151> 1999-12-01

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cag gat ccc ett atc act ttg act ccc atc att ggt ett gac atc tgg 480
Gln Asp Pro Leu Ile Thr Leu Thr Pro Ile Ile Gly Leu Asp Ile Trp
145 150 155 160

gag cac gct ttc tac etc cag tac aag aat gtc aag cct gat tac ett 528
Glu His Ala Phe Tyr Leu Gln Tyr Lys Asn Val Lys Pro Asp Tyr Leu
165 170 175

gcc get ttc tgg aac gtc tgc aac ttt get gag get cag cga agg ttt 576
Ala Ala Phe Trp Asn Val Cys Asn Phe Ala Glu Ala Gln Arg Arg Phe
180 185 190

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Asp Ala Ala Val Lys Ala
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Glu Pro Tyr Ile Ser Lys Glu Ile Met Ile Leu His His Ser Lys His
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His Gln Thr Tyr Val Thr Asn Leu Asn Ala Ala Ile Gln Ala Phe Ser
35 40 45

Gln Thr Asn Asp Ile Lys Ala Gln Ile Ala Leu Gln Ser Ala Leu Lys
50 55 60

Phe Asn Gly Gly Gly His Ile Asn His Ser Leu Phe Trp Lys Asn Met
65 70 75 80

Ala Pro Ala Asp Ser Ala Asp Ala Lys Leu Thr Glu Gly Ser Leu Lys
85 90 95

Thr Ala Ile Asp Lys Asp Phe Gly Ser Phe Glu Glu Phe Lys Lys Lys
100 105 110

Phe Asn Thr Ala Thr Leu Gly Val Gln Gly Ser Gly Trp Gly Trp Leu
115 120 125

Gly Tyr Asn Thr Ala Thr Lys His Leu Glu Ile Ala Thr Thr Ala Asn
130 135 140

Gln Asp Pro Leu Ile Thr Leu Thr Pro Ile Ile Gly Leu Asp Ile Trp
145 150 155 160

Glu His Ala Phe Tyr Leu Gln Tyr Lys Asn Val Lys Pro Asp Tyr Leu
165 170 175

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180 185 190

Asp Ala Ala Val Lys Ala
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<212> DNA

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Val Lys Thr Ser Glu Gly Asn Trp Asp Phe Val Gly Asn Asn Thr Pro
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atc ttt ttc ttg aga gac cca gcc aag ttt ccg atc ttc att cac acc 144
Ile Phe Phe Leu Arg Asp Pro Ala Lys Phe Pro Ile Phe Ile His Thr
35 40 45

cag aag agg aac ccg cag aca aac tct aaa gac aag gac gct ttc tgg 192
Gln Lys Arg Asn Pro Gln Thr Asn Ser Lys Asp Lys Asp Ala Phe Trp
50 55 60

gac tac cta tcc caa aac ccc gag tcc gtg cat cag gtg ctg cac ctg 240
Asp Tyr Leu Ser Gln Asn Pro Glu Ser Val His Gln Val Leu His Leu
65 70 75 80

ttc agt gat cga gga acc cct gct tct tac cga cac atg cat ggt tac 288
Phe Ser Asp Arg Gly Thr Pro Ala Ser Tyr Arg His Met His Gly Tyr
85 90 95

tct gga cac acc ttc aag atg gtc aac agg aac ggt gac tgg aat tat 336
Ser Gly His Thr Phe Lys Met Val Asn Arg Asn Gly Asp Trp Asn Tyr
100 105 110

gtc cag att cac atg cgc acc gat cag ggt gtc aag act cac acc aat 384
Val Gln Ile His Met Arg Thr Asp Gln Gly Val Lys Thr His Thr Asn
115 120 125

gaa gag gct tgc aaa ctc gac gcc tcc aat ccc gat tca aac gga gac 432
Glu Glu Ala Ser Lys Leu Asp Ala Ser Asn Pro Asp Ser Asn Gly Asp
130 135 140

gac ttg ttc gac gca atc aag aat gga gac ttc cct agc tgg acg gtt 480
Asp Leu Phe Asp Ala Ile Lys Asn Gly Asp Phe Pro Ser Trp Thr Val
145 150 155 160

cag gta cag gta atg tct cct gag cag gcc cag aag ttc aga tac aac 528
Gln Val Gln Val Met Ser Pro Glu Gln Ala Gln Lys Phe Arg Tyr Asn
165 170 175

att ctg gat ctc acc aag gtc tgg tcc cac aag gag ttc cca ctt agg 576
Ile Leu Asp Leu Thr Lys Val Trp Ser His Lys Glu Phe Pro Leu Arg
180 185 190

acg att gga aag ttc act ttg aac cga aac gtg gat aac tat ttc gca 624
Thr Ile Gly Lys Phe Thr Leu Asn Arg Asn Val Asp Asn Tyr Phe Ala
195 200 205

gag gtt gaa cag ctc gcc ttt gct cct tcc cat ctg cct cct gga atc 672

Glu Val Glu Gln Leu Ala Phe Ala Pro Ser His Leu Pro Pro Gly Ile
210 215 220

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<211> 238

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<213> Phaffia rhodozyma

<400> 9

Ser Gly Ser Ser Asp Thr Ala Arg Asp Pro Arg Gly Phe Ser Leu Lys
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20 25 30

Ile Phe Phe Leu Arg Asp Pro Ala Lys Phe Pro Ile Phe Ile His Thr
35 40 45

Gln Lys Arg Asn Pro Gln Thr Asn Ser Lys Asp Lys Asp Ala Phe Trp
50 55 60

Asp Tyr Leu Ser Gln Asn Pro Glu Ser Val His Gln Val Leu His Leu
65 70 75 80

Phe Ser Asp Arg Gly Thr Pro Ala Ser Tyr Arg His Met His Gly Tyr
85 90 95

Ser Gly His Thr Phe Lys Met Val Asn Arg Asn Gly Asp Trp Asn Tyr
100 105 110

Val Gln Ile His Met Arg Thr Asp Gln Gly Val Lys Thr His Thr Asn
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Glu Glu Ala Ser Lys Leu Asp Ala Ser Asn Pro Asp Ser Asn Gly Asp
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Asp Leu Phe Asp Ala Ile Lys Asn Gly Asp Phe Pro Ser Trp Thr Val
145 150 155 160

Gln Val Gln Val Met Ser Pro Glu Gln Ala Gln Lys Phe Arg Tyr Asn

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		Thr	Leu	Asn
		Arg	Asn	Val
		Asp	Asn	Tyr
		Phe	Ala	
	195	200	205	
Glu	Val	Glu	Gln	Leu
		Ala	Phe	Ala
		Pro	Ser	His
		Leu	Pro	Pro
		Gly	Ile	
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 <223> Description of Artificial Sequence:Sod1(sense
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26

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<210> 14

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<223> Description of Artificial Sequence:Sod47 (sense
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<210> 15

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<223> Description of Artificial Sequence:Sod48

(antisense primer for the construction of
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ggtacctgta ctggaggtag aaagcg

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<210> 16

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<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:Sod2 (sense
primer for cloning of CAT gene)

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mgnttytena cngtnggngg nga

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<210> 17

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<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:Cat5 (antisense
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<400> 17

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23



SEQUENCE LISTING

<110> HOSHINO, Tatsuo

OJIMA, Kazuyuki

SETOGUCHI, Yutaka

<120> PROCESS FOR THE MANUFACTURE OF CAROTENOIDS AND
BIOLOGICALLY USEFUL MATERIALS THEREOF

<130> C38435/111694

<140> 09/727,855

<141> 2000-12-01

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<170> PatentIn version 3.1

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<212> DNA

<213> Phaffia rhodozyma

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<213> Phaffia rhodozyma

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<213> Phaffia rhodozyma

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<222> (1)..(666)

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 65 70 75 80

acc cag gtt aag ctt cag tct gct ctc aag ttc aac gga gga gga cac 288
 Thr Gln Val Lys Leu Gln Ser Ala Leu Lys Phe Asn Gly Gly Gly His
 85 90 95

atc aat cac tct ctg ttc tgg aag aac ttg gct ccc tat gga tcc gag 336
 Ile Asn His Ser Leu Phe Trp Lys Asn Leu Ala Pro Tyr Gly Ser Glu
 100 105 110

gag gct acc ctc tct gaa gga cct ctc aag aag gct atc gag gaa tct 384
 Glu Ala Thr Leu Ser Glu Gly Pro Leu Lys Lys Ala Ile Glu Glu Ser
 115 120 125

ttt ggt tct ttc gag gcc ttc aag aag aag ttc aac gct gac acc gct 432
 Phe Gly Ser Phe Glu Ala Phe Lys Lys Lys Phe Asn Ala Asp Thr Ala
 130 135 140

gct gtc caa gga tcc gga tgg ggc tgg ctt ggc ttg aac ccg ctt act 480
 Ala Val Gln Gly Ser Gly Trp Gly Trp Leu Gly Leu Asn Pro Leu Thr
 145 150 155 160

aag aag ctg gaa gtc acc acg acc gcc aac cag gac cct ctg ctt act 528
 Lys Lys Leu Glu Val Thr Thr Thr Ala Asn Gln Asp Pro Leu Leu Thr
 165 170 175

cac att cct atc atc gga gtt gac atc tgg gag cac gct ttc tac ctt 576

His Ile Pro Ile Ile Gly Val Asp Ile Trp Glu His Ala Phe Tyr Leu
180 185 190

cag tac aag aac gtc aag cct gac tat ctc gct gct gtt tgg tcc gtt 624
Gln Tyr Lys Asn Val Lys Pro Asp Tyr Leu Ala Ala Val Trp Ser Val
195 200 205

atc aac tac aag gag gca gag gcc cga ttg cag gct gct ctc taa 669
Ile Asn Tyr Lys Glu Ala Glu Ala Arg Leu Gln Ala Ala Leu
210 215 220

<210> 5

<211> 222

<212> PRT

<213> Phaffia rhodozyma

<400> 5

Met Ser Val Arg Ala Ser Leu Ser Ser Val Ser Arg Gln Thr Phe Val
1 5 10 15

Ala Pro Ala Ala Phe Gln Ile Arg Ala Lys His Thr Leu Pro Glu Leu
20 25 30

Pro Tyr Ala Tyr Asp Ala Leu Glu Pro Ser Ile Ser Lys Glu Ile Met
35 40 45

Thr Leu His His Thr Lys His His Gln Thr Tyr Val Asn Gly Leu Asn
50 55 60

Ala Ala Glu Glu Ser Tyr Ser Ala Ala Val Gly Lys Glu Asp Val Leu
65 70 75 80

Thr Gln Val Lys Leu Gln Ser Ala Leu Lys Phe Asn Gly Gly Gly His

85

90

95

Ile Asn His Ser Leu Phe Trp Lys Asn Leu Ala Pro Tyr Gly Ser Glu
100 105 110

Glu Ala Thr Leu Ser Glu Gly Pro Leu Lys Lys Ala Ile Glu Glu Ser
115 120 125

Phe Gly Ser Phe Glu Ala Phe Lys Lys Lys Phe Asn Ala Asp Thr Ala
130 135 140

Ala Val Gln Gly Ser Gly Trp Gly Trp Leu Gly Leu Asn Pro Leu Thr
145 150 155 160

Lys Lys Leu Glu Val Thr Thr Thr Ala Asn Gln Asp Pro Leu Leu Thr
165 170 175

His Ile Pro Ile Ile Gly Val Asp Ile Trp Glu His Ala Phe Tyr Leu
180 185 190

Gln Tyr Lys Asn Val Lys Pro Asp Tyr Leu Ala Ala Val Trp Ser Val
195 200 205

Ile Asn Tyr Lys Glu Ala Glu Ala Arg Leu Gln Ala Ala Leu
210 215 220

<210> 6

<211> 597

<212> DNA

<213> Phaffia rhodozyma

<220>

<221> CDS

<222> (1)..(594)

<223>

<400> 6

atg gct cct tac act ctt ccc gac ctt cct tac gct tac gat gcc ttg 48
Met Ala Pro Tyr Thr Leu Pro Asp Leu Pro Tyr Ala Tyr Asp Ala Leu
1 5 10 15

gag cct tac atc tct aag gaa atc atg atc ctt cac cac tcc aag cac 96
Glu Pro Tyr Ile Ser Lys Glu Ile Met Ile Leu His His Ser Lys His
20 25 30

cat cag act tac gtc acc aac ctc aac gcc gct atc cag gct ttc tcc 144
His Gln Thr Tyr Val Thr Asn Leu Asn Ala Ala Ile Gln Ala Phe Ser
35 40 45

cag acc aat gac atc aag gcc cag atc gct ctt cag agc gct ctc aag 192
Gln Thr Asn Asp Ile Lys Ala Gln Ile Ala Leu Gln Ser Ala Leu Lys
50 55 60

ttc aac gga gga gga cac atc aac cac tcc ctc ttc tgg aag aac atg 240
Phe Asn Gly Gly Gly His Ile Asn His Ser Leu Phe Trp Lys Asn Met
65 70 75 80

gct cct gcc gac tct gct gat gcc aag ctc acc gag gga tgc ctc aag 288
Ala Pro Ala Asp Ser Ala Asp Ala Lys Leu Thr Glu Gly Ser Leu Lys
85 90 95

act gcc atc gac aag gac ttt gga tcc ttc gag gag ttc aag aag aag 336
Thr Ala Ile Asp Lys Asp Phe Gly Ser Phe Glu Glu Phe Lys Lys Lys
100 105 110

ttc aac act gct act ctc ggt gtc cag gga tct gga tgg gga tgg ctc 384
Phe Asn Thr Ala Thr Leu Gly Val Gln Gly Ser Gly Trp Gly Trp Leu
115 120 125

gga tac aac acc gct acc aag cac ctc gag atc gcc acc acc gcc aac 432
Gly Tyr Asn Thr Ala Thr Lys His Leu Glu Ile Ala Thr Thr Ala Asn

130

135

140

cag gat ccc ctt atc act ttg act ccc atc att ggt ctt gac atc tgg 480
Gln Asp Pro Leu Ile Thr Leu Thr Pro Ile Ile Gly Leu Asp Ile Trp
145 150 155 160

gag cac gct ttc tac ctc cag tac aag aat gtc aag cct gat tac ctt 528
Glu His Ala Phe Tyr Leu Gln Tyr Lys Asn Val Lys Pro Asp Tyr Leu
165 170 175

gcc gct ttc tgg aac gtc tgc aac ttt gct gag gct cag cga agg ttt 576
Ala Ala Phe Trp Asn Val Cys Asn Phe Ala Glu Ala Gln Arg Arg Phe
180 185 190

gal gct gct gtc aag gct taa 597
Asp Ala Ala Val Lys Ala
195

<210> 7

<211> 198

<212> PRT

<213> Phaffia rhodozyma

<400> 7

Met Ala Pro Tyr Thr Leu Pro Asp Leu Pro Tyr Ala Tyr Asp Ala Leu
1 5 10 15

Glu Pro Tyr Ile Ser Lys Glu Ile Met Ile Leu His His Ser Lys His
20 25 30

His Gln Thr Tyr Val Thr Asn Leu Asn Ala Ala Ile Gln Ala Phe Ser
35 40 45

Gln Thr Asn Asp Ile Lys Ala Gln Ile Ala Leu Gln Ser Ala Leu Lys
50 55 60

Phe Asn Gly Gly Gly His Ile Asn His Ser Leu Phe Trp Lys Asn Met
65 70 75 80

Ala Pro Ala Asp Ser Ala Asp Ala Lys Leu Thr Glu Gly Ser Leu Lys
 85 90 95

Thr Ala Ile Asp Lys Asp Phe Gly Ser Phe Glu Glu Phe Lys Lys Lys
 100 105 110

Phe Asn Thr Ala Thr Leu Gly Val Gln Gly Ser Gly Trp Gly Trp Leu
 115 120 125

Gly Tyr Asn Thr Ala Thr Lys His Leu Glu Ile Ala Thr Thr Ala Asn
 130 135 140

Gln Asp Pro Leu Ile Thr Leu Thr Pro Ile Ile Gly Leu Asp Ile Trp
 145 150 155 160

Glu His Ala Phe Tyr Leu Gln Tyr Lys Asn Val Lys Pro Asp Tyr Leu
 165 170 175

Ala Ala Phe Trp Asn Val Cys Asn Phe Ala Glu Ala Gln Arg Arg Phe
 180 185 190

Asp Ala Ala Val Lys Ala
 195

<210> 8

<211> 714

<212> DNA

<213> Phaffia rhodozyma

<220>

<221> CDS

<222> (1)..(714)

<223>

<400> 8

tcc gga agc tca gat acc gct cga gat cct cga ggt ttc tct ctt aag 48
Ser Gly Ser Ser Asp Thr Ala Arg Asp Pro Arg Gly Phe Ser Leu Lys
1 5 10 15

gtc aag acc tct gag gga aac tgg gac ttt gtc gga aac aac act ccc 96
Val Lys Thr Ser Glu Gly Asn Trp Asp Phe Val Gly Asn Asn Thr Pro
20 25 30

atc ttt ttc ttg aga gac cca gcc aag ttt ccg atc ttc att cac acc 144
Ile Phe Phe Leu Arg Asp Pro Ala Lys Phe Pro Ile Phe Ile His Thr
35 40 45

cag aag agg aac ccg cag aca aac tct aaa gac aag gac gct ttc tgg 192
Gln Lys Arg Asn Pro Gln Thr Asn Ser Lys Asp Lys Asp Ala Phe Trp
50 55 60

gac tac cta tcc caa aac ccc gag tcc gtg cat cag gtg ctg cac ctg 240
Asp Tyr Leu Ser Gln Asn Pro Glu Ser Val His Gln Val Leu His Leu
65 70 75 80

ttc agt gat cga gga acc cct gct tct tac cga cac atg cat ggt tac 288
Phe Ser Asp Arg Gly Thr Pro Ala Ser Tyr Arg His Met His Gly Tyr
85 90 95

tct gga cac acc ttc aag atg gtc aac agg aac ggt gac tgg aat tat 336
Ser Gly His Thr Phe Lys Met Val Asn Arg Asn Gly Asp Trp Asn Tyr
100 105 110

gtc cag att cac atg cgc acc gat cag ggt gtc aag act cac acc aat 384
Val Gln Ile His Met Arg Thr Asp Gln Gly Val Lys Thr His Thr Asn
115 120 125

gaa gag gct tcg aaa ctc gac gcc tcc aat ccc gat tca aac gga gac 432
 Glu Glu Ala Ser Lys Leu Asp Ala Ser Asn Pro Asp Ser Asn Gly Asp
 130 135 140

gac ttg ttc gac gca atc aag aat gga gac ttc cct agc tgg acg gtt 480
 Asp Leu Phe Asp Ala Ile Lys Asn Gly Asp Phe Pro Ser Trp Thr Val
 145 150 155 160

cag gta cag gta atg tct cct gag cag gcc cag aag ttc aga tac aac 528
 Gln Val Gln Val Met Ser Pro Glu Gln Ala Gln Lys Phe Arg Tyr Asn
 165 170 175

att ctg gat ctc acc aag gtc tgg tcc cac aag gag ttc cca ctt agg 576
 Ile Leu Asp Leu Thr Lys Val Trp Ser His Lys Glu Phe Pro Leu Arg
 180 185 190

acg att gga aag ttc act ttg aac cga aac gtg gat aac tat ttc gca 624
 Thr Ile Gly Lys Phe Thr Leu Asn Arg Asn Val Asp Asn Tyr Phe Ala
 195 200 205

gag gtt gaa cag ctc gcc ttt gct cct tcc cat ctg cct cct gga atc 672
 Glu Val Glu Gln Leu Ala Phe Ala Pro Ser His Leu Pro Pro Gly Ile
 210 215 220

gag ccc tcg aac gat ccc gtc ctt cag gct cga cta ttc tcc 714
 Glu Pro Ser Asn Asp Pro Val Leu Gln Ala Arg Leu Phe Ser
 225 230 235

<210> 9

<211> 238

<212> PRT

<213> Phaffia rhodozyma

<400> 9

Ser Gly Ser Ser Asp Thr Ala Arg Asp Pro Arg Gly Phe Ser Leu Lys
 1 5 10 15

Val Lys Thr Ser Glu Gly Asn Trp Asp Phe Val Gly Asn Asn Thr Pro
20 25 30

Ile Phe Phe Leu Arg Asp Pro Ala Lys Phe Pro Ile Phe Ile His Thr
35 40 45

Gln Lys Arg Asn Pro Gln Thr Asn Ser Lys Asp Lys Asp Ala Phe Trp
50 55 60

Asp Tyr Leu Ser Gln Asn Pro Glu Ser Val His Gln Val Leu His Leu
65 70 75 80

Phe Ser Asp Arg Gly Thr Pro Ala Ser Tyr Arg His Met His Gly Tyr
85 90 95

Ser Gly His Thr Phe Lys Met Val Asn Arg Asn Gly Asp Trp Asn Tyr
100 105 110

Val Gln Ile His Met Arg Thr Asp Gln Gly Val Lys Thr His Thr Asn
115 120 125

Glu Glu Ala Ser Lys Leu Asp Ala Ser Asn Pro Asp Ser Asn Gly Asp
130 135 140

Asp Leu Phe Asp Ala Ile Lys Asn Gly Asp Phe Pro Ser Trp Thr Val
145 150 155 160

Gln Val Gln Val Met Ser Pro Glu Gln Ala Gln Lys Phe Arg Tyr Asn
165 170 175

Ile Leu Asp Leu Thr Lys Val Trp Ser His Lys Glu Phe Pro Leu Arg
180 185 190

Thr Ile Gly Lys Phe Thr Leu Asn Arg Asn Val Asp Asn Tyr Phe Ala
195 200 205

Glu Val Glu Gln Leu Ala Phe Ala Pro Ser His Leu Pro Pro Gly Ile
210 215 220

Glu Pro Ser Asn Asp Pro Val Leu Gln Ala Arg Leu Phe Ser
225 230 235

<210> 10

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Sod1 (sense primer for cloning of SOD genes)

<220>

<221> misc_feature

<222> (1)..(23)

<223> n or X = A, C, G or T

<400> 10

aarcaycayc aracntaygt naa

23

<210> 11

<211> 23

<212> DNA

<213> Artificial Sequence

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<223> Sod4 (antisense primer for cloning of SOD genes)

<220>

<221> misc_feature

<222> (1)..(23)

<223> n or X = A, C, G or T

<400> 11

gcccancng anccytgnac ncc

23

<210> 12

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Sod14 (sense primer for the construction of SOD1--disrupting plas
mid)

<400> 12

ggtacctccg atgataggaa tgtgag

26

<210> 13

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Sod15 (antisense primer for the construction of SOD1-disrupting plasmid)

<400> 13

gaattcagtt caacggagga ggacac

26

<210> 14

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Sod47 (sense primer for the construction of SOD2-disrupting plasmid)

<400> 14

gaattcggag gaggacacat caaccg

26

<210> 15

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Sod48 (antisense primer for the construction of SOD2-disrupting plasmid)

<400> 15

ggtacctgta ctggaggtag aaagcg

26

<210> 16

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Sod2 (sense primer for cloning of CAT gene)

<220>

<221> misc_feature

<222> (1)..(23)

<223> n or X = A, C, G or T

<400> 16

mgnttytcna cngtnggngg nga

23

<210> 17

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Cat5 (antisense primer for cloning of CAT gene)

<220>

<221> misc_feature

<222> (1)..(23)

<223> n or X = A, C, G or T

<400> 17

ckrtgnckyt gngtrtcngg rta

23